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DECAY OF BRAZIL NUTS

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(WITH PLATES VIII–XII AND THREE FIGURES)

Introduction

Brazil nuts, Para nuts, Cream nuts, etc., are the seeds of *Bertholletia nobilis* Miers and *B. excelsa* Humb. and Bonpl. The nuts are harvested in the months of January, February, and March, when the heavy pericarps containing the seeds fall to the ground. They are collected and transported from the forests to the seaports at a time of year when heat and moisture favor fungous growth, and often a cargo reaches New York 30 per cent spoiled. The United States Bureau of Chemistry holds that nuts are adulterated food if more than 15 per cent are spoiled, and requires that such nuts be shelled before being placed on the market. In spite of these measures, however, Brazil nuts reach the consumer containing from 10 to 25 per cent of spoiled nuts. There were 43,076,348 pounds of Brazil and Cream nuts shipped into the United States in 1919 (7). It is probable that half of this amount, or 21,538,174 pounds, were retailed in the shell. It is conservative to estimate the loss through spoiled nuts at 10 per cent of this amount, or 2,153,817 pounds, an approximate money loss, at 40 cents per pound, of $861,526.80, which falls directly upon the consumer. Brazil nuts do not become rancid very readily, and for this reason they are not placed in cold storage during warm weather as are most other nuts. They are heaped in piles in supposedly dry and often very

hot rooms where, when moisture is present, fungous growth is favored.

There is a wide difference in shell porosity of Brazil nuts, and a positive correlation between fungous infection and shell porosity has been demonstrated. Two two-pound samples of Brazil nuts purchased at two different grocery stores were tested for porosity of shell as follows. The nuts were taken one at a time and dipped first in 95 per cent alcohol to prevent the collection of surface bubbles, and then plunged two or more inches beneath the surface

TABLE I

Percentage spoiled	Very porous		Slightly porous		Least porous		Spoiled in sample
	Cracked	Spoiled	Cracked	Spoiled	Cracked	Spoiled	
	Sample I						
100	4	4	.....	.....	.....	.....	.....
34	.....	.....	26	9	.....	.....	.....
12	.....	.....	.....	.....	49	6	.....
25, in sample	.....	.....	.....	.....	.....	.....	19
	Sample II						
50	2	1	.....	.....	.....	.....	.....
38	.....	.....	21	8	.....	.....	.....
7	.....	.....	.....	.....	70	5	.....
15, in sample	.....	.....	.....	.....	.....	.....	14

of hot water contained in a tall beaker. The heat-expanded air arose in bubbles from the pores of the shell. Table I shows the results obtained. The conclusion to be drawn from these data is that the most porous nuts are not necessarily spoiled, but readily become infected when conditions favor infection, while the least porous nuts are much less subject to infection. It is quite possible that so long as the water content of the nut is sufficient to support fungous growth, nuts with very porous shells may be entered and spoiled if storage temperature is favorable. Such infections probably account for the high percentage of spoiled Brazil nuts bought of retailers whose wholesale patrons have scrupulously complied with the ruling of the Bureau of Chemistry when the nuts were purchased at port.

Although the use of nuts as foods and confections has recently become extended and general, there is but little concerning nut diseases in the literature, and studies of the diseases affecting the nuts only for the most part have been superficial. MANGIN (17) described a "black rot" of chestnuts caused by *Harziella castanae* Bain., and found it to cause a 26 per cent loss of nuts gathered late in the season. VON IVANOFF (31), in studying *Trichothecium roseum* Link, found it in pure state on the kernels of *Corylus avellana* and *Pinus cembra*, and this, with RAND'S (23) work on *Coniothyrium caryogenium* Rand, is the only serious investigation of nut parasites that has been made. MARTZ (18) reports a species of *Cephalothecium* on pecans in Florida. KUHL (13) isolated *Aspergillus flavus* Mont. from Brazil nuts, but his description of both disease and fungus is meager.

A few parasites of nut plants cause diseases of the nuts themselves. The most serious disease of this kind is that produced by *Pseudomonas juglandis* Pierce, which, according to SMITH (27), attacks the nuts as well as other growing parts, and "a nut in such cases is deformed in shape . . . . and the kernel . . . . is only poorly developed." PIERCE (21) says that in young nuts the kernel is destroyed. Chestnuts are affected by *Endothia parasitica* (Murr.) A. and A. RUMBOLD (24) says that the hyphae of this parasite spread throughout the kernel. The kernel spot of pecan produced by *Coniothyrium caryogenium* Rand has incidently been studied by TURNER (30), and by RAND (23). In addition to these studies, there have been some reports on storage results (6, 29), and MCMURRAN (16) mentions what he considers a non-parasitic disease, the "black-pit" of pecan.

The aim of the present investigation was to isolate and identify as many as possible of the more important fungi and bacteria causing deterioration of Brazil nuts. Seven distinct organisms have been isolated, studied, and their etiological relation to the nut deterioration demonstrated. The remainder of the paper comprises the methods of study and descriptions of the organisms isolated.

Methods

The nuts studied were obtained from two wholesale firms in Chicago and from retail grocery stores in Champaign and Urbana,

Illinois, and were purchased at different times during the year 1919–20. Each nut was superficially examined and the shell carefully removed by cracking it with a hammer. The diseased nuts were dropped, shell and all, into suitable sterilized glass dishes, one nut in each dish. A number was assigned to each, but only those diseases which were most prevalent and which presented the most conspicuous diagnostic features were selected for study.

A preliminary examination was made of thin razor sections of diseased tissue mounted in water or xylol, in order to discover whether fungi or bacteria were present, and if so, to ascertain their general relation to the host tissues. If this examination showed any single species of organism to be predominant, isolations were made either by direct transfer to cornmeal agar plates, or by dilution plating as the case required. These isolations were from both exterior and interior portions of the nut, and when from the interior were carried out in the following manner. The nut meat was cut into with a flamed scalpel and carefully broken apart. A central portion of about 4 sq. mm. area was carefully removed with a flamed scalpel from one of the newly exposed surfaces, and discarded. In the center of the cavity thus made small pieces of diseased tissue were loosened with the point of the scalpel, and immediately carried in a sterilized loop to the surface of cornmeal agar plates.

Following isolation, the next step was to determine whether the fungi isolated were responsible for the various diseased conditions. Two methods were used; first, pieces of mycelium or a few spores were placed on sterile kernels contained in sterilized one-inch test tubes; and second, pieces of mycelium or a few spores were placed upon strips of sterile nut meat, 50 $\mu \times 5 \times 10$ mm., which were contained in tubes of sterile water, one strip on the side of the tube just above the surface of the water and the other in the water. By the first of these methods the rotting power of the parasite was made evident within a few weeks by the softening of the whole mass. With the second method results were obtained more quickly by more or less complete dissolution of the very thin sections employed. The following media were used in the case of every organism.

CORNMEAL AGAR.—This was prepared as recommended by SHEAR (**26**), except that the medium ready for filtration was poured into precipitation cones and autoclaved. After solidifying, the precipitated dirt at the apex of the inverted cone was removed and the clean agar melted and tubed.

BRAZIL NUT AGAR.—Fifty grams of Brazil nut kernels which were free of, or easily freed from, their inner seed coats were ground in a nut grinder (a Russwin no. 1 Food Cutter was used), and steeped for one hour at from 58° to 60° C. in 1000 cc. of distilled water, counterpoised, and filtered. Fifteen grams of powdered or crude agar was added and the mixture boiled for ten minutes, counterpoised, poured into precipitation cones, and autoclaved at 15 pounds for fifteen minutes. After solidification the agar was removed from the glass cone and placed on a clean sheet of paper. After removing the sediment the dirt-free agar was returned to the precipitation cone and again autoclaved. The resolidified agar cone was in two distinct layers, and the translucent layer was the one used.

NUT PLUGS.—It was found possible, by flaming a scalpel after each stroke, to cut out nut plugs of considerable size which were free from contamination. The kernels from which such plugs were to be cut were placed in a 2 to 1000 solution of mercuric chlorid where they remained for thirty minutes. They were taken from the solution one at a time, held by one end between thumb and finger, and shaped by cutting away a thin layer with a sharp scalpel, flamed after each stroke. When the plug was finished it was cut off after placing it within the mouth of the reclining one-inch test tube. Nut plugs made in this way remained uncontaminated for several months.

NUT STRIPS.—Strips of nut meat, 50 $\mu \times 5 \times 10$ mm., were cut on a microtome and preserved in absolute alcohol. When used they were taken from the alcohol with sterilized forceps and placed in sterile water in a Petri dish. From this they were removed with a sterilized loop and placed in test tubes containing sterile water, as already described.

AUTOCLAVED RICE.—This medium was made by placing two or three grams of rice and twice the volume of water in test tubes and autoclaving.

MICROTOME SECTIONS.—These were made in order to show the morbid histology in comparison with the normal histology. Because of the oil content of the nut, ether was found to be the best killing and fixing agent. The ether was replaced after three days with chloroform, and the ordinary schedule for imbedding with this reagent followed (**2**). After sectioning it was found that the oil content had not been sufficiently lessened, and that without its removal a distinct view of the structures could not be secured. To obviate the difficulty the sections were fixed to the slide, treated with xylol, xylol and absolute alochol, absolute alcohol, and then flooded four or five minutes with ether. The slide was held horizontally between thumb and finger, and the dissolved fats collected on the under side of the slide, from which they were wiped off before placing the slide in 95 per cent alcohol. The slide was kept in each of the 95, 85, and 70 per cent alcohols for about five minutes, and then flooded with Pianese IIIb for 15 minutes (**32**), washed with distilled water, 70, 85, and acidulated 95 per cent alcohol, 95, 100 per cent alcohol, 100 per cent alcohol and xylol, xylol, and mounted in balsam. The stain shows the host tissue in red and the fungus in green.

Two of the fungi produced pycnidia which were sectioned for study. These were taken from cultures on cornmeal agar, killed and fixed with chromacetic fixing fluid, and stained with Bismark brown, following the usual schedule for this stain. The pycnidia were removed from the culture on a strip of agar, usually about $1 \times 2 \times 4$ mm. in size, which remained intact throughout the process and served well in the orientation of the specimens in the imbedding dish.

FREEHAND SECTIONS.—It was occasionally found necessary to stain razor sections made for the preliminary study of the diseased tissue. The sections were cut as thin as possible and placed in a watch glass contained in a Petri dish. The watch glass was then filled with ether and the Petri dish closed. When the ether had evaporated, 95 per cent alcohol was poured over the sections and allowed to stand for ten or fifteen minutes. This was followed with 85, 70, and 50 per cent alcohols for five minutes each. The sections were then transferred to slides prepared with albumen

fixative, flooded with water, and allowed to stand over night. They were stained with Pianese IIIb, or with jod grün-erythrosin in the following manner. They were placed in 95 per cent alcohol for five minutes, flooded with jod grün (1 per cent solution in 95 per cent alcohol) for thirty minutes, washed with 95 per cent alcohol, then absolute alcohol, flooded with 1 per cent solution of erythrosin in clove oil for forty-five minutes, washed with absolute alcohol, cleared with carbol-turpentine clearer, and finally washed in xylol and mounted in balsam. This proved to be the most satisfactory of any method tried for staining mycelium in the tissue.

PROTEOLYTIC ENZYMES.—The Brazil nut agar serves well to show the presence or absence of certain extracellular proteolytic enzymes. The proteid precipitates to which the opacity of the agar is due are digested by the enzymes, and a transparent halo, which enlarges as the thallus or colony enlarges, surrounds the growth. All the organisms studied were tested for the presence of these enzymes. The enzymes were precipitated from cornmeal broth in which an *Actinomyces* or a *Bacillus*, the two most active enzyme producers, was grown. The broth was poured into Piorkowski culture flasks to a depth of 0.25 inch, about 200 cc. being required for each flask, and inoculated. After ten days the culture was filtered through paper and enough 95 per cent alcohol added to the filtrate to make 80 per cent alcohol. Three days later a fluffy white precipitate had collected at the bottom of the precipitation cones, and the excess alcohol was siphoned off (**12**). From 25 to 50 cc. of absolute alcohol was added to the precipitate and immediately filtered. Before the precipitate had become dry it was again washed with 50 cc. of absolute alcohol, and while still moist was removed to a desiccator containing sulphuric acid, and allowed to remain there for two days. The hard, gray material was then scraped from the paper to be redissolved in sterile water when used.

MORPHOLOGY OF BRAZIL NUT.—The kernel of the Brazil nut, as it is ordinarily removed on cracking the shell, is covered with a thin, dry coat which may be quite loose or may adhere very tenaciously. The embryo within, principally radicle, is completely

enveloped with a layer of endosperm 40 to 50 μ in thickness, and as reported by YOUNG (**35**) is "plainly differentiated into cortical and medullary tissues separated by a layer of procambium along which rudimentary vascular bundles are arranged at intervals." There is a single, somewhat irregular layer of epidermal cells just beneath the endosperm, and within 5 mm. of the distal end are the two very minute, unequal cotyledons which "measure about 750 by 175 μ" (**1, 25**). The cortical and medullary cells are similar in shape and size, and are largely filled with oil and proteid bodies. The endosperm cells, arranged with the long diameter at right angles to a median plane, are especially rich in proteids (**28**). The procambium cells with the long diameter at right angles to that of the endosperm cells contain few or no proteid grains (fig. 1).

The outer seed coat or shell is made up of two layers; the outer with its crinkled surface is light brown and softer in texture than the inner layer, which is dark brown and has a glazed inner surface. In the angles of the shell this layer seems to be of two layers which divide, leaving spaces filled with still another tissue that is lighter in color and softer in texture than the outer of the shell layers. In the micropylar angles of the seed is a narrow cavity. Such cavities are termed by BERG (**1**) the "loculi spurii in testa," and extend the entire length of the shell. This open channel probably serves as the usual entrance of the parasites of the nut (fig. 1).

The tissues of the seed, taken in order, beginning with the shell are: (1) outer seed coat in two distinct layers, with a softer tissue filling the triangularly prismatic corners; (2) the thin inner seed coat which may or may not adhere to the kernel; (3) the endosperm layer, two cells thick; (4) epidermis, a single-celled layer; (5) cortex, of large storage cells; (6) procambium layer, generally three cells thick; (7) the medullary tissue, of large storage cells.

Diseases of the Brazil nut

1. BLACK CRUST

GENERAL DESCRIPTION.—Fully 5 per cent of all diseased Brazil nuts are affected with black crust, but there is no external indication of their condition, since the shells are normal in color and the

weight is the same as that of sound nuts. When the shell is removed the kernel presents a dull black appearance which, if the whole nut is affected, reminds one of a large sclerotium. A cross-section of the diseased kernel shows the blackened portion to consist of a thin layer, 100–250 μ in thickness, apparently having no connection with the tissues beneath, which, aside from their light brown color and their pronounced nut odor, appear to be normal

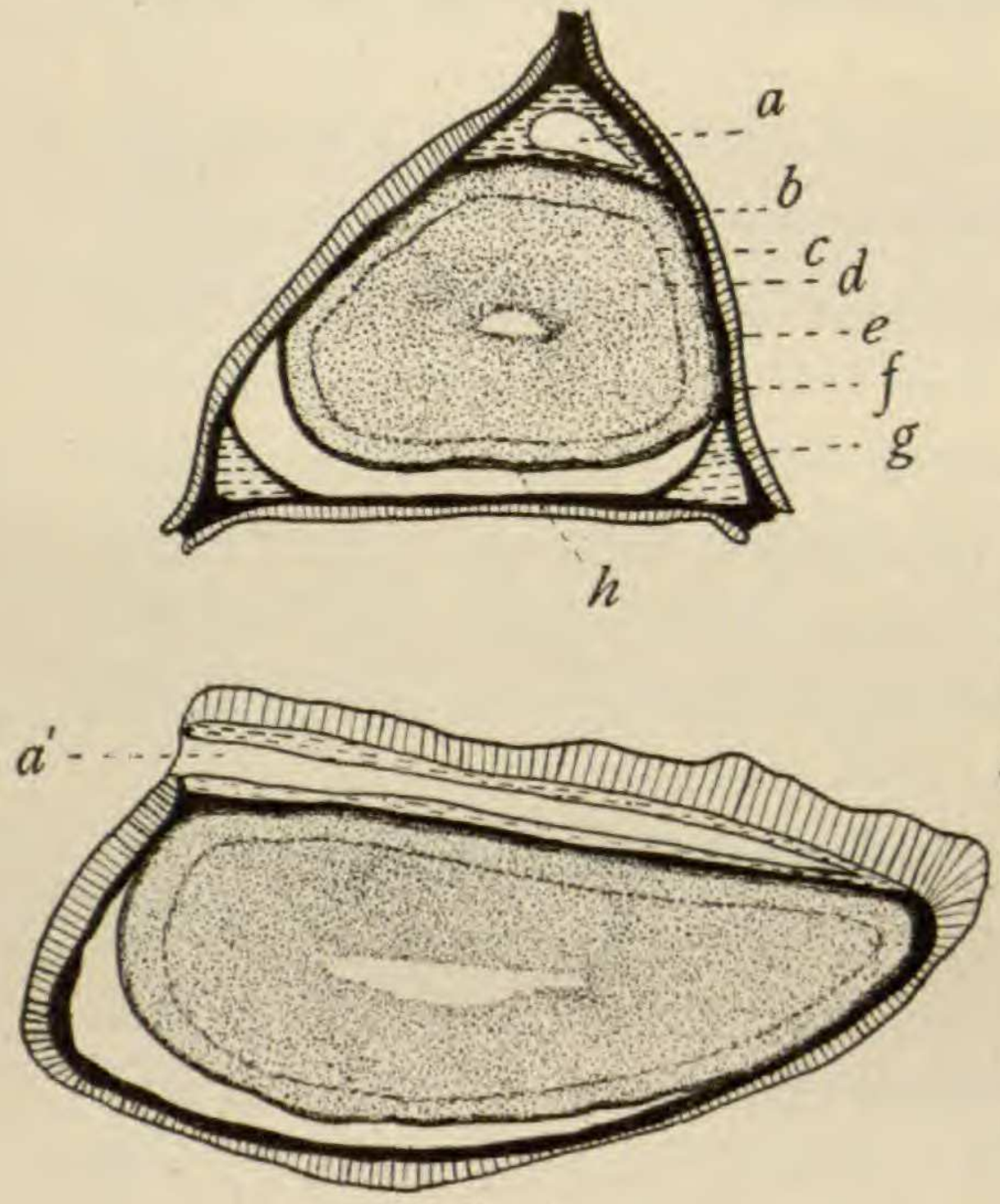

FIG. 1

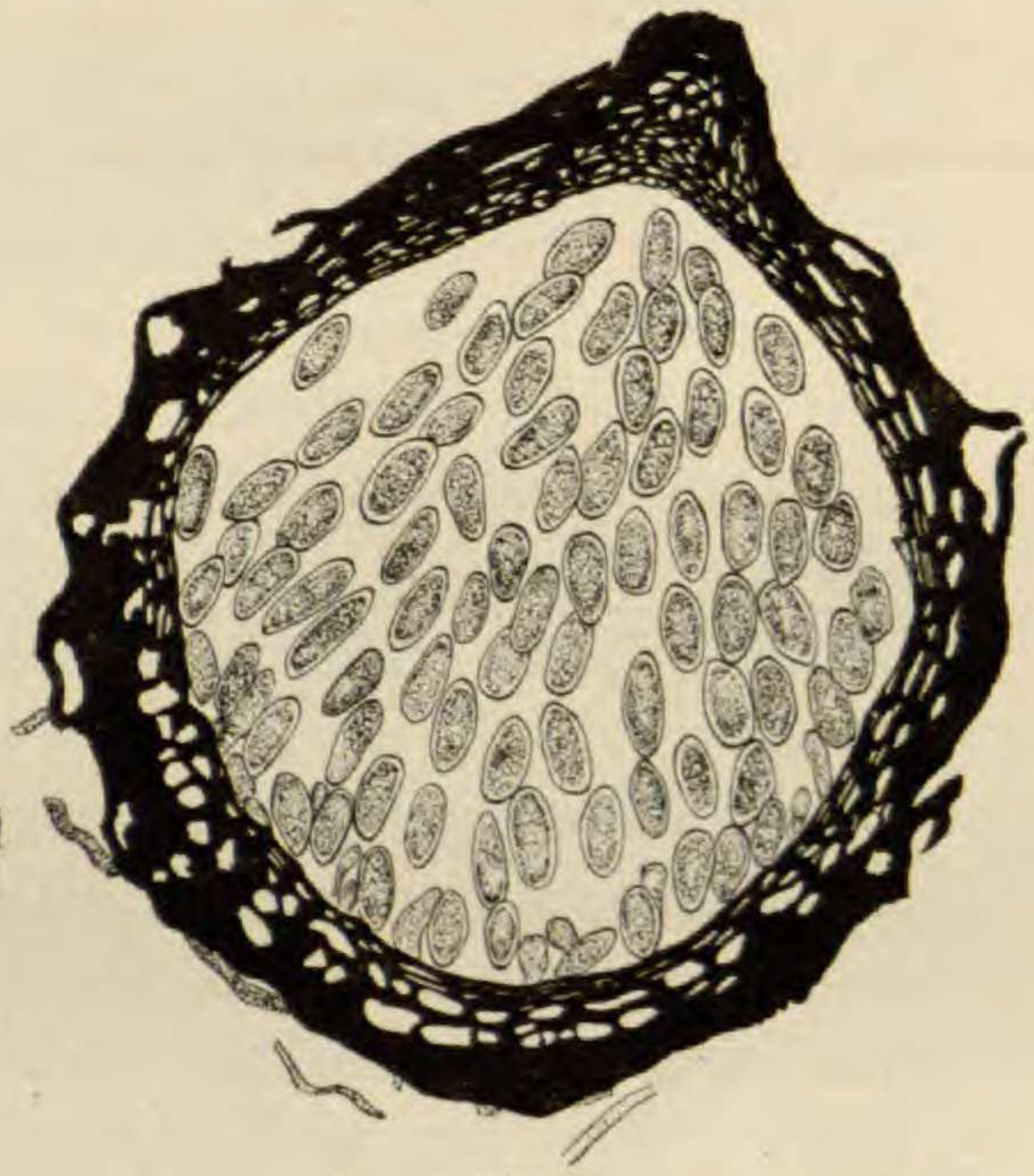

FIG. 2

FIGS. 1, 2.—Fig. 1, Diagrammatic drawings of cross and longitudinal sections of Brazil nut: *a* and a^1, locule in testa; *b*, endosperm layer; *c*, procambium layer; *d*, medullary tissue; *e*, outer layer of outer seed coat; *f*, inner layer of outer seed coat; *g*, soft tissue filling corners of shell; *h*, inner seed coat; fig. 2, pycnidium with immature spores, *Pellioniella macrospora* n. sp.; ×500.

(figs. 34–36). The diseased nut meats are frequently found covered with various fungi, chiefly *Penicillium* or *Aspergillus*, with black crust under the mold. A study of microtome sections shows that the mycelium is in the endosperm layer, the affected cells of which are hypertrophied (figs. 34, 35). The cortical cells of the radicle immediately beneath are not parasitized, but their contents are markedly changed. The proteid grains are almost or quite lacking in the epidermal and outer layers of the normal cortex, while in diseased nuts there is a superabundance of small proteid grains in

this region (figs. 34, 35). As it is possible to find nuts seemingly free from any other organism, the black crust fungus is easily isolated. Small pieces of tissue taken aseptically from immediately below the crust on direct plating gave pure cultures.

MORPHOLOGY.—The mycelium on cornmeal agar is of two kinds, that made up of cells which are longer than wide, and that with cells either nearly globular or wider than they are long (figs. 18, 19). The long-celled type predominates, both in the aerial and the submerged mycelium, except near pycnidia, where the shorter cells are most in evidence. The long cells are 14–32 μ in length by 3.5–14 μ in width, the short ones measuring 10–18 μ in diameter. Both types are thick-walled and black when mature, and both have granular contents (figs. 19, 20). In autoclaved rice and in the black crust of diseased nuts the hyphal cell is transformed until the hyphae suggest chains of conidia (figs. 14, 20). These cells are black, 10–15×5–8 μ in size, and contain one or two guttulae. They readily break away from the hyphae and function as spores.

Pycnidia are produced sparingly, and only along the border of a thallus where it comes in contact with another thallus, either of the same or of some other species. No pycnidia were found on diseased nuts or on any of the cultures except those on cornmeal agar plates. They are black, smooth, globose-conical, beaked, and 150–350 μ in diameter. The beak is 100–250 μ in length (figs. 2, 21).

The spores are borne at the base of the pycnidial cavity on short, hyaline, often septate conidiophores which are interspersed with narrow strap-shaped, hyaline, continuous paraphyses that are from one to six times as long as the conidiophores, the conidiophores being 5–14 μ in length by 3–5 μ in width (fig. 13). The spores are at first hyaline, unicellular, 26–36×14–20 μ in size and irregular in shape, but with maturity they become sooty black, striated, uniseptate, regular in shape and uniform in size, being 28×14 μ (fig. 15).

CULTURE CHARACTERS.—On cornmeal agar plates the fungus grows at the rate of 0.5–0.7 mm. per day at room temperature. The thallus is at first milk white, and the margin of it remains uncolored so long as it is increasing in size. After five or six days

the central portion becomes green, and a few days later turns sooty black. The thallus then is made up of three concentric rings, the outer white, the next green, and the innermost black (fig. 11). As the thallus ages it shows marked zonation, and becomes entirely black when growth ceases. Aerial mycelium is produced on all parts of the thallus, but is most luxuriant in the central area. On Brazil nut agar the growth is very much slower than on cornmeal agar, usually 0.3 mm. per day at room temperature, and the entire thallus remains hyaline. On nut plugs the growth was very weak, but a crust similar to that of naturally diseased nuts was formed after three months. On autoclaved rice the growth was vigorous, and several characteristic color reactions were noted (fig. 8). Eight days after inoculation: aerial mycelium snow white with line of Antique Green[1] below; rice grains in contact with glass, white bordered with Cerulian Blue; all interstices with greenish tints. After fifteen days: aerial mycelium white with lower border line Prussian Blue, almost black; contacts of grains with glass white bordered with Prussian Blue; interstices purple tinged.

The hyaline immature spores as well as the black mature ones germinated readily. The immature spores occasionally germinated in ten minutes after planting, while more than an hour is required for the germination of the mature spores, but the germ tubes of the mature ones soon outstrip those of the immature (figs. 16, 17). There is no change in either spore, except a slight swelling in germination, the immature spore remaining unicellular. This phenomenon of the germination of immature as well as mature spores has been pictured by HIGGINS (11) for a related species.

TAXONOMY.—The morphological characters of the fungus are those of *Pellioniella* Sacc., but according to SACCARDO (25) there is but one species in the genus, *P. deformans* Penz. and Sacc., whose spore measurements are a little more than half those of the Brazil nut parasite. The fungus, therefore, is given the name *Pellioniella macrospora*.

[1] The nomenclature used in describing colors throughout these investigations is that given in ROBERT RIDGEWAY'S Color standards and color nomenclature, published by the author, Washington, D.C. 1912.

Pellioniella macrospora, n. sp.—Pycnidia sparse, smooth, carbonaceous, globose-conical, beaked, 150–350 μ in diameter, beak 100–250 μ in length. Conidiophores at base of pycnidial cavity, hyaline, often septate, 5–15×3–5 μ. Paraphyses hyaline, strap-shaped, continuous, 5–50 μ. Immature conidia hyaline, unicellular, irregular, 26–36×14–20 μ; mature conidia sooty black, striated, uniseptate, regular, 28×14 μ.

HABITAT.—Parasitic on endosperm of seed of *Bertholletia nobilis* Miers and *B. excelsa* Humb. and Bonpl.

2. WHITE MOLD

GENERAL DESCRIPTION.—White mold is not so common as black crust, and is probably responsible for less than 1 per cent of the Brazil nut decay, but it is a real factor in this loss. The diseased nut is normal in external appearance, but is below normal in weight. When cracked, the white, fluffy mycelium is seen to cover the entire kernel, but soon after exposure the hyphae collapse and the yellowed endosperm becomes visible through the mycelial mass. A pronounced musty odor arises from the newly shelled nut, but the taste of the diseased meat has nothing to distinguish it. A cross-section of the nut kernel shows three typical features of the disease: (1) the white moldy covering; (2) the endosperm layer, sulphur-yellow in color and more than twice as thick as in the normal nut; and (3) irregular cracks and cavities in the radicle, all filled with white mycelium and spores. The mycelium penetrates to the center of the radicle. The hyphae in the tissue are very tenuous, less than 2 μ in diameter, and are usually so closely associated with the cell walls of the host tissue as to make a study of them *in situ* very difficult, but the cell walls of the diseased nut are penetrated by them. The fungus was isolated as described, but spore dilutions made by touching a sterile loop to the mycelial mass in the internal check of the kernel gave pure cultures also.

MORPHOLOGY.—Mycelium taken from the nut, from nut plugs, and from other media was uniform in character. The following description is of mycelium taken from cornmeal agar plates. The cells measure 20–70×3.5–10.5 μ, and are hyaline with granular contents and guttulae. Anastomosis of cells is of frequent occur-

rence, especially in the submerged hyphae, while in the aerial mycelium simple loops and coils are common (fig. 24). The hyphae are unconstricted at the septa, and unbranched cells are of quite uniform size throughout their length. Cells bearing branches are swollen at the points from which the branches arise.

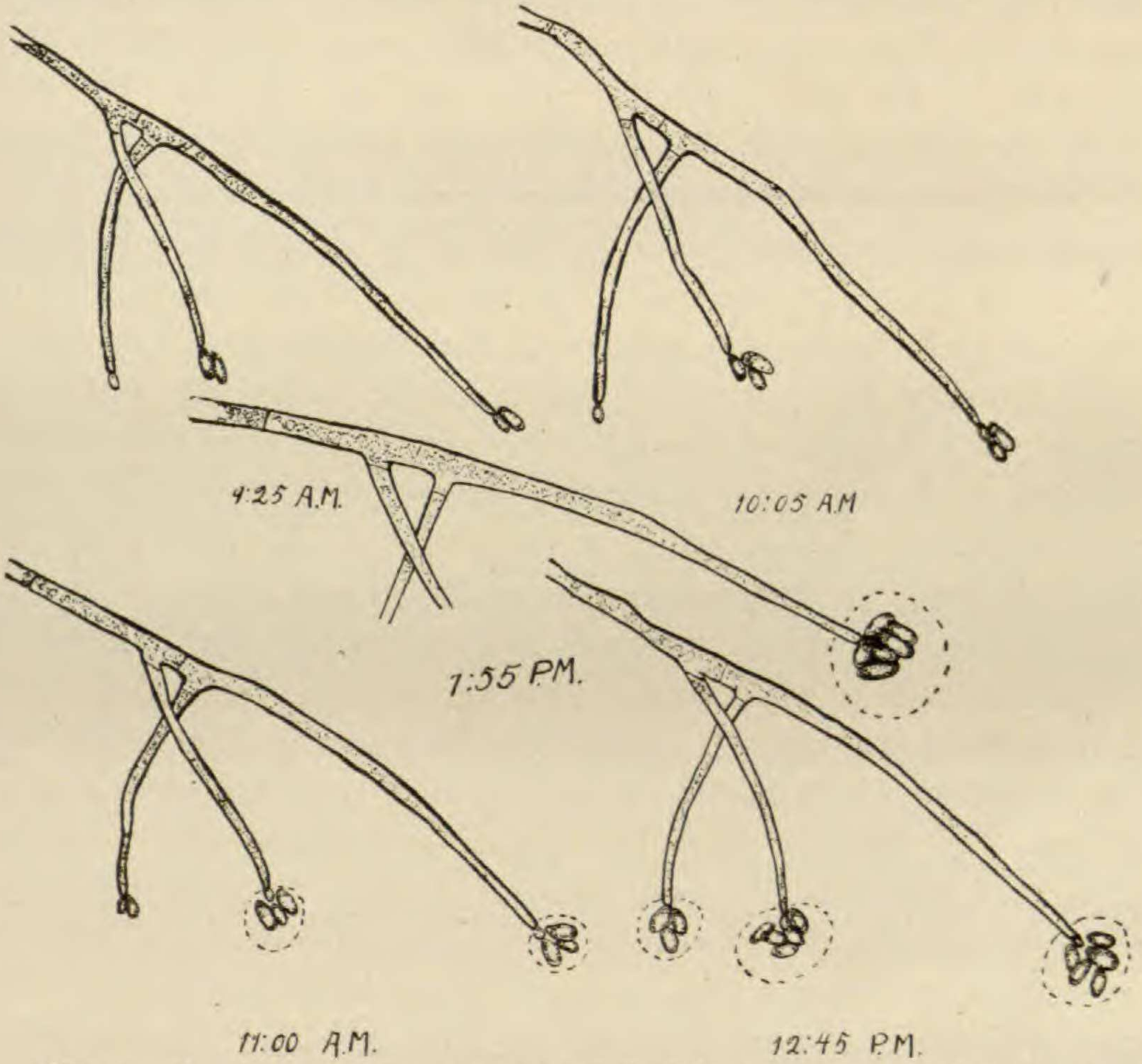

FIG. 3.—Development of spore mass of *Cephalosporium bertholletianum* n. sp.; dotted line shows size of water drop surrounding mass; ×100.

The conidiophores, 50–90 μ in length, are in most instances simple branches from either a principal filament or from its branches. They are spindle-like, with rounded tips from which the spores are cut off (fig. 3). The single-celled, oblong-elliptical, hyaline conidia are 8–12×3.5–54 μ in size, and contain two guttulae (fig. 23). They collect, as they are produced, in a spherical mass at the end of the conidiophore. A drop of water surrounds the spore mass after the third spore arrives (fig. 3).

CULTURE CHARACTERS.—On cornmeal agar the fungus grows rather rapidly, 0.5–1.0 cm. daily at room temperature. The thallus is very regular and is distinctly zonated after reaching a diameter of 4 or 5 cm. There is a dense growth of white aerial mycelium on the older portions of the thallus. On Brazil nut agar the characters are as previously stated, except that the growth is a little more rapid, and a halo 3 mm. wide, due to the digestion of the solid proteids, surrounds the thallus. On nut plugs the fungus grows luxuriantly and destroys the nut meat without giving off any appreciable odor. A large amount of fluffy mycelium is the characteristic feature of its growth on nut plugs as it is on the nut in the shell. On autoclaved rice the growth is vigorous and a pink tinge appears in the medium after two days. After ten days four colors are distinguished; where the rice is in contact with the glass in the older portions, Ochraceous Buff, in the younger, Venetian Pink; interstices between the grains are filled with mycelium through which a Jasper Pink color shows, in the older portions; in the younger portions it is Light Hortense Violet.

The nut strip above the water is soon covered with the dense mycelium, and is appreciably shrunken within five days. The strip in the water remains intact, but the water is soon filled with the mycelium which makes its way upward from the strip at the bottom. In hanging drop the spores germinate with a single germ tube, which in the most vigorous, at room temperature, may attain the length of the spore in two hours after planting. Spore production in hanging drop at room temperature proceeds at the rate of about one spore per hour per conidiophore. The conidiophore lengthens and increases in diameter as the conidia are cut off at the end. The process is very like spore production of *Trichothecium* as described by LINDAU (**14**).

TAXONOMY.—The fungus evidently belongs to *Cephalosporium*, but none of the species of this genus as reported by SACCARDO (**25**) has characters sufficiently like the Brazil nut parasite to permit it to be classified as one of them. *C. fructigenum* McAlp. (**15**) has spores of almost identical shape, size, and appearance, but it has knobbed conidiophores and oblong spore masses which are not present in this species, which therefore is described as new.

Cephalosporium bertholletianum, n. sp.—Conidiophores hyaline, simple or dichotomously branched, 50–90 μ long, 2- to 4-septate; spore mass globular; conidia hyaline, unicellular, oblong-elliptical, guttulate, 6–12×3.5–5 μ, ends obtuse.

HABITAT.—On radicle of seed of *Bertholletia nobilis* Miers and *B. excelsa* Humb. and Bonpl., causing decay.

3. DRY ROT

GENERAL DESCRIPTION.—The shell of the nut affected by dry rot is mottled, but of somewhat lighter shade in its darkest areas than the shells of normal nuts, and the weight is much below normal. The cracked shell appears to be filled with a kernel which adheres more closely to the shell than is usual, but which is so similar in color and general appearance to that of sound nut kernels that it might easily pass casual observation as such, although in reality it is merely a mass of mycelium. Small pieces of mycelium taken from this mass swell to approximately twice their size when placed in water. Under the microscope the hyphae were seen to be irregularly branched and septate. No conidiophores were seen, but what appeared to be unicellular elongated conidia of greatly varying length were occasionally found.

MORPHOLOGY.—The hyphae which make up both the aerial and the submerged mycelium are irregularly branched, and more or less constricted at the septa. The cells are 14–90 μ long by 3.5–11 μ wide, hyaline with granular contents and guttulae (fig. 44). Anastomosis frequently occurs, especially in older thalli, when spores falling on the medium germinate, producing a tube which unites with the cell of an older hypha, another germ tube, or another spore (figs. 29, 48).

The simple conidiophores are borne at any place along the hyphal strand, seldom more than two being produced by a single cell. Branched conidiophores are rare. Thalli resulting from direct planting of mycelium taken from the diseased nut produce but few conidiophores, and rarely more than single-celled conidia. Conidia from transferred cultures are from 1- to 8-celled, subcylindrical, slightly sickle-shaped, without pedicel, and conical at base (fig. 42).

No perithecia were found, but sclerotia were formed on autoclaved rice. These are dark gray and 500–1000 μ in diameter. Terminal chlamydospores are produced on 60-day old cultures. They are globular or oblong, with an average mean diameter of 14 μ, and with a scarcely perceptible yellow tinge (fig. 45).

CULTURE CHARACTERS.—Pure cultures are easily obtained by directly planting pieces of mycelium taken from the innermost portion of the mycelial mass. On cornmeal agar the rate of growth averages 0.5 cm. daily at room temperature. The thallus is arachnoid and regularly zonated, the zones averaging 0.5 cm. in width. Aerial mycelium covers the entire thallus but is most luxuriant in the central area, and there it is tufted. On Brazil nut agar the growth is more dense and a little more rapid than on cornmeal agar, its rate being from 0.7 to 1.0 μ at room temperature. An extra-cellular, proteolytic enzyme is secreted, causing a halo of 3–5 mm. in width in the medium surrounding the thallus. The aerial mycelium is more luxuriant on this agar than on the other.

The fungus grows vigorously on nut plugs, so that in a few days the plugs are enveloped with the snow-white mycelium, while a putrid odor is exhaled. After two or three months the plugs are completely reduced and only a mycelial mass remains. There is no color change on autoclaved rice until it shrinks away from the tube, when it is Maize Yellow. The fluffy, white, aerial mycelium surmounts the rice column and covers its sides as the growth proceeds downward. Apparently complete destruction of the rice is accomplished within two months. On carrot plugs the growth is not so rapid as on other media, but is marked by an abundance of white aerial mycelium. It is without color change. On the strip of nut meat above the water the growth is vigorous, and if the water surface remains near enough to the strip it is destroyed within eight or ten days. The strip in the water often appeared to be intact when it was not, the mycelium retaining the outline. It was probably destroyed as soon as the strip above.

In hanging drop the conidia begin germinating after three or four hours at room temperature, but many of them require twenty-four hours or more. Seldom more than two cells of a spore germinate, but frequently one cell produces two germ tubes (fig. 46). It often happens that spores are united by a short germ tube. Occa-

sionally four or five conidia are connected in this way (fig. 47), resulting, as is clearly shown by drop cultures, from germination succeeded by anastomosis (fig. 48). In all cultures the dense mycelium collects and retains water enough to germinate the 1- and 2-celled spores, and their germ tubes anastomose readily with the first cells, conidial or hyphal, with which they come in contact. It is often difficult to distinguish between conidiophores bearing conidia and conidia anastomosed to hyphal cells with a short germ tube (fig. 44).

TAXONOMY.—The fungus is a species of *Fusarium* which, according to WOLLENWEBER'S (34) scheme of classification, belongs to the section Eupionnotes: chlamydospores present; perithecia unknown; conidia subcylindrical, sickle-shaped; base without pedicel, conical; terminal chlamydospores.

4. ASPERGILLUS DECAY

GENERAL DESCRIPTION.—Brazil nuts attacked by *Aspergillus* may give no external indication of their internal condition except in the most advanced stages of the disease, when the weight of the nut is appreciably lowered. The kernel shrinks, often cracks open, and is always covered with a mass of dark brown spores. The odor of the diseased nut is strongly rancid with a putrid taint; the taste is at first sour, later very bitter. Occasionally nuts that are merely discolored have this same taste. KUHL (13) states that Brazil nuts affected with *Aspergillus flavus* Mont. are poisonous, and that the discoloration caused by this fungus is so slight that it does not prevent their being eaten. Both his observations and my own indicate that the disease, although present, may often escape notice, and that it is really far more prevalent than it appears to be under superficial examination. Nuts in advanced stages of the disease, however, occur less frequently than black crust. The mycelium of the fungus penetrates the tissues to the center of the nut, and when there is a central locule, appears as a white mold on the walls of the locule. When the diseased kernels crack open, a mass of spores fills the locular space.

MORPHOLOGY.—The mycelium consists of irregularly branched hyphae which are slightly constricted at the septa (fig. 31). The cells are 20–65 × 3.5–11 μ, with granular contents of a faint greenish

tint. Conidiophores varying from ten to several hundred microns in length arise at irregular intervals from the hyphae. The shortest of these have little or no filamentous part, but consist merely of the head and sterigmata (fig. 28). Sterigmata are also borne singly and in groups of from two to four on the hyphal cells (fig. 27). The heads of the conidiophores measure 10–20 μ in diameter, and the sterigmata, from two to many per head, are 10–12 × 5–7 μ. The globular, echinulated conidia are of different shades of yellow, and 5–10 μ in diameter, but the predominant size is 7 μ (fig. 29).

CULTURE CHARACTERS.—On cornmeal agar the rate of growth varies from 0.3 to 1.0 mm. daily at room temperature, and after forty-eight hours the central portion of the thallus shows the forming spore clusters in Light-Buff. The spore masses become darker with age until Lemon-Chrome is finally reached. On Brazil nut agar the growth is very similar to that on cornmeal agar, but with a halo 1 to 2 mm. in width, showing the presence of an extracellular, proteolytic enzyme surrounding the thallus. The color of the spore mass at maturity is from Orange-Cinnamon to Mikado-Brown. On nut plugs the growth is rapid, and a gas with the odor of carbon bisulphide is evident. The color of the spore mass is Primrose-Yellow at first, Honey-Yellow to Tawny-Olive at maturity. At the end of two or three months all that remains of the nut plug is a mass of partially disintegrated cell walls in a mass of mycelium.

The growth on autoclaved rice is vigorous, with spore masses forming within forty-eight hours. There is little change in the color of the medium except for the development of a slight greenish-yellow tint below the spore mass. The color of the spore mass changes from Oil-Yellow to Orange-Cetrine. The odor of a 30-day old culture is very like that of cider vinegar. The nut strip above the water is entirely covered with spore masses within three days, but only about one-fourth of it is destroyed before it becomes too dry to support the fungus. A luxuriant growth of mycelium arises from the strip in the water, and usually the strip is destroyed before fifteen days.

TAXONOMY.—A culture of this species was sent to CHARLES THOM, and the following excerpt is taken from his reply dated January 29, 1920:

The organism belongs to the general group in which we are trying to separate three lines, the *Aspergillus oryzae-flavus* line, the *Aspergillus wentii* section, and the one which has been designated by KITA as *Aspergillus tamari*. This one, from the examination today, would appear to belong to the section containing *A. tamari*. Whether it is safe to identify it under an existing name or not would be doubtful.

5. BACTERIAL DECAY

GENERAL DESCRIPTION AND MORPHOLOGICAL CHARACTERS.—When Brazil nuts are affected by this bacterial decay, the shell is black and greasy, and usually exhales a rancid odor. When the shell is cracked open the remains of the kernel are found as a white mass which ordinarily fills only a small portion of the shell cavity. Microscopic examination of fragments of the refuse shows numerous bacterial spores, but usually no vegetative forms and no fungi. When dilution plates were made from the decayed residue, one spore-bearing organism largely predominated.

The vegetative cells of the organisms in cornmeal broth are rod-shaped, rounded at the ends, vigorously motile, and usually single but often in chains of from two to six individuals. The rods measure 2.5–5.0 $\mu \times 0.8$–1.2 μ. Spores are formed within forty-eight hours in one end of the vegetative cells. When the cells are stained by LOEFFLER'S method, the organism is found to have numerous long, peritrichiate flagella; stained with LOEFFLER'S methylene blue the protoplasm is seen to be granular with from two to four darkened patches which are unevenly distributed, usually giving a banded effect, although often the bands are oblique as well as horizontal (fig. 38). The organism stains readily with methylene blue, Gentian violet, and carbol-fuchsin, but it is Gram negative.

When sterile nut plugs were inoculated with the bacillus from pure culture, they were reduced in about fifteen days to an oily mass which, in all essential characters, was like the remains of the nut kernels in the natural cases of nut decay. Dilution plates made from nut meats that had decayed, following pure culture

inoculation, showed only one type of colony, and this proved to consist of the organism with which the plugs had been inoculated. The organism had no appreciable effect on the nut strip above the water, and the strip in the water was only very slowly decomposed, but strips in cornmeal bouillon were completely destroyed within ten to fifteen days. The organism grows best in the presence of air, as the colonies on all plated media and stab culture show, but deep lenticular colonies (fig. 40), and colonies next to the glass (fig. 39) in agar plates, as well as the faint line of growth along the stab, indicate that it is a facultative anaerobe.

While none of the usual tests for particular enzymes was made, the reactions in different culture media indicate the production of diastase, invertase, rennet, and pepsin. In Brazil nut agar plates there is formed a transparent halo about the colony, and as the opacity of the agar is due to the presence of solid proteid matter (**20**), the halo results from the digesting of these proteids. There is an abundant secretion of the protease which makes the halo, as the diameter of the transparent area is from two to three times that of the colony itself. This enzyme was precipitated as already described, and drops of a water solution of the dried precipitate placed on Brazil nut agar plates. A transparent area as large as the drop of solution was formed in a plate 2 mm. thick in from two to three hours.

The organism seems to be an undescribed one, and a complete description of it will be given in a separate paper.

6. Actinomyces decay

General description and morphology.—Empty shells that are intact and still retain their normal color are occasionally found among Brazil nuts. When these shells are cracked open a characteristic musty odor is evident, and the inner shell wall is seen to be covered with pinkish velvety pustules that are from one to several millimeters in diameter. Water mounts of pieces of a pustule show tenuous, mycelial-like strands, or chains of spores which readily stain with carbol-fuchsin. The filaments are not long but branch, and the mass is so bound together by the branches that it is quite impossible to separate entire filaments from the mass.

The filaments are never entirely straight nor yet very crooked, and chains of spores are usually contained in the free ends (fig. 37). No spirals were found on any of the media. The diameter of the filaments varies from 1.0 to 1.3 μ, and the oblong spores measure 1.6×0.8 μ.

The germination of spores was studied with an oil immersion lens, in a hanging drop prepared as follows. A thin film of synthetic agar was spread on a thin cover-glass, and a loop full of a dilute spore suspension placed on the agar film. This was inverted over a dry Van Tieghem cell. The water soon evaporated, leaving the spores in contact with the agar, where their germination was easily studied and camera lucida drawings made. According to DRECHSLER (**9**), *Actinomyces* spores produce from one to four germ tubes, "the approximate number being more or less characteristic of the species." This species produces one and two germ tubes which often branch directly on leaving the conidium (fig. 36).

The organism was studied in the manner suggested by CONN (**3**) and WAKSMAN (**33**), and the media were made in accordance with directions given by WAKSMAN (**33**). The following culture characters were noted:

CULTURAL CHARACTERS.—1. Synthetic agar: room temperature, after ten days: growth densely compact but thalli small, at first white, but after ten days Pale Pinkish Buff; aerial mycelium white and dense; soluble pigment none.

2. Calcium malate-glycerin agar: growth spreading and not zonated, bordered by submerged mycelial bands of varying width, pearl white; aerial mycelium short, loose, and pearl white; soluble pigment.

3. Glucose agar: growth luxuriant, color same as in synthetic agar, thallus conspicuously zonated; aerial mycelium white to Pale Pinkish Buff, powdery; soluble pigment none.

4. Glycerin agar: growth densely compact, not zonated, Pale Pinkish Buff; aerial mycelium powdery, white; soluble pigment none.

5. Brazil nut agar: growth rapid, densely compact with wide margin of submerged mycelium, white to Pale Pinkish Buff;

aerial mycelium dense, white; soluble pigment none; enzymatic zone three to four times the diameter of thallus.

6. Cornmeal agar: growth dense but zonated, Pale Pinkish Buff; aerial mycelium powdery; soluble pigment none.

7. Egg albumin agar: growth thin, conspicuously zonated, Pale Pinkish Buff; aerial mycelium powdery, Pale Pinkish Buff; soluble pigment none.

8. Nut plugs: growth vigorous, Pale Pinkish Buff; aerial mycelium powdery, white; medium not completely destroyed, but much shrunken and blackened.

9. Autoclaved rice: growth vigorous, Pale Pinkish Buff; aerial mycelium 2 cm.; almost completely destroyed in sixty days.

10. Potato plugs: growth vigorous, crumpled, Pale Pinkish Buff; aerial mycelium abundant, at first white, later Pale Pinkish Buff; medium with no change in color, much reduced in size in two months.

11. Carrot plugs: growth at first slow, appearing after five days, crumpled and dense, Pale Pinkish Buff; aerial mycelium powdery, at first white, later Pale Pinkish Buff; medium darkened near the growth, no change in color in other regions, much shrunken.

12. Brazil nut bouillon: growth surface pellicle, snow-white; aerial mycelium white, powdery; medium somewhat clarified.

13. Nut strips: growth slight on strip above water, and none on strip in water; no growth on surface of water.

BIOCHEMICAL FEATURES.—The proteolytic enzyme which makes the halo in Brazil nut agar plates was the only one studied, but the growth reactions in different media were taken to indicate the probable production of several other enzymes, diastase and invertase especially. The proteolytic enzyme was isolated by precipitation, as previously described, and its proteolytic power tested by placing drops of a water solution of the dried precipitate on Brazil nut agar plates. Transparent areas the size of the drops developed in from two to three hours, depending upon the thickness of the agar plates.

TAXONOMY.—The organism is an *Actinomyces* which, according to WAKSMAN'S key, belongs in division *B*, "no soluble pigment produced on gelatin or other protein media," and in section *I*,

"species strongly proteolytic; gelatin liquefied rapidly, milk clotted and peptonized rapidly." No species given in this division and section, however, has the characteristics of the one found in Brazil nut shells. It is therefore given the name of *Actinomyces brasiliensis*.

Actinomyces brasiliensis, n. sp.—Straight, branched hyphae 1.0–1.3 μ in diameter; spores borne in chains in free ends of hyphae, oblong, 1.6×0.8 μ; growth Pale Pinkish Buff on all agars except calcium malate-glycerin, on which it is white; zonated on glucose, cornmeal, and egg albumin agars; aerial mycelium on all media, white to Pale Pinkish Buff; no soluble pigment formed.

HABITAT.—Parasitic on kernels of Brazil nuts.

7. PHOMOPSIS DECAY

GENERAL DESCRIPTION.—Only one nut was found affected with *Phomopsis* decay, but because of its striking diagnostic features the fungus was isolated and studied. There was no external indication of the diseased condition, but the kernel of the nut was rich brown, with a few black specks near one end. The odor of the nut was pleasant and the taste agreeable. Stained hand sections showed that the mycelium of the fungus had penetrated into the radicle to considerable depth.

MORPHOLOGY.—The mycelium was tenuous, septate, and at first hyaline, but soon became brown or smoke colored. According to DIEDICKE (8), the form of the pycnidia is greatly varied. In the Brazil nut species several of the forms pictured by DIEDICKE were observed, but the one most commonly met with was mammiform, with a wartlike protuberance. The irregular pycnidial cavity so common to the genus was frequently observed, but a regular cavity was the rule. Two forms of spores were present in all pycnidia examined (fig. 50), and as is customary, the *Phoma* type will be designated as *A*, the filamentous as *B* spores. The *B* form did not germinate in hanging drop, a fact supporting the statement made by GROVE (10) that these may or may not be spores. When they fail to germinate they are probably what SACCARDO (25) took them to be, conidiophores, which according to GROVE "become more curved than when *in situ*." The *A*

spores are oblong-elliptical, hyaline, guttulate, and measure $5–7 \times 1.7–3.5\ \mu$. The *B* spores are filiform, usually hook-shaped, hyaline, continuous, and measure from $17–24.5 \times 2–3.5\ \mu$.

CULTURE CHARACTERS.—The fungus grew well on all media used. On cornmeal agar the thallus was circular and without zonations. A loose aerial mycelium covered the entire thallus, and numerous pycnidia varying in size were scattered over the surface of the plate. The pycnidia appeared simultaneously with the brown color, which was usually noticed after five or six days. On Brazil nut agar the growth was similar to that on cornmeal agar. The clear halo formed in the agar plate barely exceeded the size of the thallus. On autoclaved rice a brown or smoky color, due to the mycelial growth, was noticeable, but no color change occurred in the medium. Nut plugs were soon covered with a brown mycelium which later became almost black. The surface was soon covered with black, wartlike pycnidia, and the entire mass when cut through suggested a dried sponge. The odor was similar to that of very rancid nuts. The fungus made no growth on nut strips above the water, but a dense mass of mycelium, filled with black strands, developed on the strips in the water. These strips retained their form, but the cessation of mycelial growth, which occurred between ten and fifteen days after inoculation, marked the time of nutrient exhaustion.

TAXONOMY.—The fungus is a typical *Phomopsis* which approaches *P. aucubicola* Grove more nearly than any other described species. *A* spores are shorter, and this, coupled with the fact that it occurs on an unrelated host, necessitates describing it as new.

Phomopsis bertholletianum, n. sp.—Pycnidium dark brown, mammiform, with wartlike protuberance, irregular in shape and size, varying from 0.1 to 1.0 μ; conidiophores filiform, hyaline, continuous, 15–20 μ long, often indistinguishable from *B* spores. *A* and *B* spores present, *A* spores oblong-elliptical, hyaline, guttulate, $5–7 \times 1.7–3.5\ \mu$; *B* spores filiform, usually hook-shaped, hyaline, continuous, $17–24.5 \times 2–3.5\ \mu$.

HABITAT.—Parasitic on kernels of Brazil nuts.

8. Bitter rot

Figure 4 shows a part of a Brazil nut affected with bitter rot, and fig. 49 shows spores of the fungus, two of which have conidiophores attached. Neither the spores nor the mycelium was viable, and time prevented more than a superficial examination being made. The fungus is apparently a *Myxosporium*.

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LITERATURE CITED

1. Berg, Otto, Bertholletia excelsa Hb. et Bpl., Martii Flora Braziliensis. 14:478. 1859.
2. Chamberlain, C. J., Methods in plant histology. University of Chicago Press. Chicago. 1915.
3. Conn, H. J., The possible function of *Actinomyces* in soil. Jour. Bact. 1:197. 1916.
4. ———, Soil flora studies, V. *Actinomyces* in soil. N.Y. Agric. Expt. Sta. Tech. Bull. 60. 1917.
5. Chester, F. D., A manual of determinative bacteriology. Macmillan Co. New York. 1914.
6. Corbet, L. C., Cold storage. W.Va. Agric. Expt. Sta. Bull. 75. 1901.
7. Department of Commerce. Brazil and Cream nuts. Monthly Summary of Foreign Commerce of the United States. December, 1919.
8. Diedicke, H., Die Gattung *Phomopsis*. Ann. Myc. **9**:8. 1911.
9. Drechsler, C., Morphology of the genus *Actinomyces*. Bot. Gaz. **67**:65–83; 147–168. 1919.
10. Grove, W. B., The British species of *Phomopsis*. Roy. Bot. Gard., Kew Bull. Misc. Inform. no. 2. 1917 (p. 49).
11. Higgins, B. B., Plum wilt, its nature and cause. Ga. Agric. Expt. Sta. Bull. 118. 1916.
12. Jones, L. R., Pectinase the cytolytic enzyme produced by *Bacillus carotovorus* and certain other soft-rot organisms. N.Y. Agric. Expt. Sta. Tech. Bull. 11. 1909.
13. Kuhl, Hugo, Über eine eigenartige Veranderung der Paranuss. Pharm. Zentralh. **51**:106. 1910.
14. Lindau, G., *Trichothecium roseum*. Rabenhorst's Kryptogamen-Flora. 8:366. 1907.
15. McAlpine, D., Fungus diseases of stone-fruit trees in Australia. Dept. Agric., Melbourne, Victoria. 1902.
16. McMurran, S. M., Diseases of trees. Amer. Nut Jour. 4:81. 1916.

17. MANGIN, M. L., La Pourriture des Chataignes. L'Aced. D'Agr. France. 4:885. 1918.
18. MARTZ, J., Diseases and insect pests of the pecan. Fla. Agric. Expt. Sta. Bull. 147. 1918.
19. MIGULA, W., System der Bacterien. Handbuch der Morphologie Entwickelungsgeschichte und Systematik der Bacterien. Verlag von Gustav Fischer, Jena. 1900.
20. OSBORNE, T. B., and CLAPP, S. H., Hydrolysis of excelsin. Amer. Jour. Phys. 19:53. 1907.
21. PIERCE, N. B., Walnut bacteriosis. BOT. GAZ. 31:272. 1901.
22. PEARSON, C. H., Uses of the Brazil nut tree. Amer. Forestry. 23:782. 1919.
23. RAND, F. V., Some diseases of pecans. Jour. Agric. Res. 1:303. 1914.
24. RUMBOLD, CAROLINE, Notes on chestnut fruits infected with the chestnut blight fungus. Phytopath. 5:64. 1915.
25. SACCARDO, P. A., Sylloge Fungorum. Vol. XVIII.
26. SHEAR, C. L., and STEVENS, NEIL E., Cultural characters of the chestnut blight fungus. U.S. Dept. Agric. B.P.I. Cir. 131. 1913.
27. SMITH, R. E., Walnut culture in California, walnut blight. Cal. Agric. Expt. Sta. Bull. 231. 1912.
28. STRASBURGER, E., Textbook of botany, 4th Eng. ed. Macmillan Co. London. 1912.
29. STUCKEY, R. P., Pecans, varieties, influence of climate, soil, stock on scion. Ga. Agric. Expt. Sta. Bull. 116. 1915.
30. TURNER, WILLIAM F., *Nezara viridula* and kernel spot of pecan. Science 47: 490. 1918.
31. VON IVANOFF, K. S., Über *Trichothecium roseum* Link als Ursache der Bitterfaule von Früchten. Zeitsch. Pfl'kheit 14:36. 1904.
32. VAUGHAN, R. E., A method for the differential staining of fungus and host cells. Ann. Mo. Bot. Gard. 1:241. 1914.
33. WAKSMAN, S. A., Cultural studies of species of *Actinomyces*. Soil Sci. 8:71. 1918.
34. WOLLENWEBER, H. W., *Ramularis, Mycosphaerella, Nectria, Calonectria*. Eine Morphologisch Pathologische Studie zur Abgrenzung von pilzgruppen mit cylindrischen und sichelformigen Konidienformen. Phytopath. 3: 197. 1913.
35. Young, W. J., A study of nuts with special reference to microscopic identification. U.S. Dept. Agric. Bur. Chem. Bull. 160.

EXPLANATION OF PLATES VIII–XII

All drawings were made with camera lucida.

PLATE VIII

FIG. 4.—Part of Brazil nut kernel affected by bitter rot; ×1.

FIG. 5.—Thalli of *Actinomyces brasiliensis* n. sp. on cornmeal agar; ×¾.

FIG. 6.—Thallus of *Actinomyces brasiliensis* n. sp. on cornmeal agar; ×2½.

FIG. 7.—Colony of *Bacillus* from Brazil nut on cornmeal agar; ×2.

FIG. 8.—*Pellioniella macrospora* n. sp. in autoclaved rice; tube on right 20 days old; tube on left 10 days old; ×1.

FIG. 9.—Brazil nut 30 days after inoculation with *Pellioniella macrospora* n. sp.; nut plug at top marks place of inoculation, and along two edges inner seed coat removed to expose blackened endosperm; ×1.

FIG. 10.—Thallus of *Actinomyces brasiliensis* n. sp. on Brazil nut agar, surrounded by transparent area in which proteids have been digested owing to secretion of proteolytic enzyme; ×1½.

FIG. 11.—Thallus of *Pellioniella macrospora* n. sp. on cornmeal agar: three zones: (1) scarcely visible outer zone of white; (2) zone of nearly same width that is green in growing thallus; (3) inner black circle; ×¾.

FIG. 12.—*Actinomyces brasiliensis* n. sp. on potato plug after 10 days; ×1.

PLATE IX

FIG. 13.—Paraphyses, immature conidia, and conidiophores of *Pellioniella macrospora* n. sp.; ×500.

FIG. 14.—Conidia-like cells of hyphae of *P. macrospora* n. sp. from diseased tissue of Brazil nut; ×500.

FIG. 15.—Mature, immature, and transitional stages in development of conidia of *P. macrospora* n. sp.; ×500.

FIG. 16.—Germination of immature conidia of *P. macrospora* n. sp., planted in same hanging drop with mature conidia shown in figure 18; ×500.

FIG. 17.—Germination of mature conidia of *P. macrospora* n. sp.; germ tubes from one to two hours longer in emerging than those of immature conidia shown in figure 17; ×500.

FIG. 18.—Hypha of *P. macrospora* n. sp., showing most common type of cell; ×500.

FIG. 19.—Hypha of *P. macrospora* n. sp. from near pycnidium; ×500.

FIG. 20.—Conidia-like cells of hyphae of *P. macrospora* n. sp., taken from culture in autoclaved rice; ×500.

FIG. 21.—Section of pycnidium of *P. macrospora* n. sp., enlarged about 450 diameters.

FIG. 22.—Hyphae of *P. macrospora* n. sp., showing two types of cells; ×500.

PLATE X

FIG. 23.—Conidia of *Cephalosporium bertholletianum* n. sp.; ×500.

FIG. 24.—Hyphae, conidiophores, and spore masses surrounded by water drops, *C. bertholletianum* n. sp.; ×500.

FIG. 25.—Germinating conidia of *C. bertholletianum* n. sp., two hours after planting; ×500.

FIG. 26.—Germinating conidia of *C. bertholletianum* n. sp., twenty hours after planting; ×500.

FIG. 27.—Hyphae of Brazil nut *Aspergillus* bearing sterigmata; ×500.

FIG. 28.—Hypha of Brazil nut *Aspergillus* bearing short stalked conidiophores; ×500.

FIG. 29.—Conidia of Brazil nut *Aspergillus;* ×500.

FIG. 30.—Mature conidiophores of Brazil nut *Aspergillus;* ×500.

FIG. 31.—Hyphae showing branching habit and anastomosis, Brazil nut *Aspergillus;* ×500.

FIG. 32.—Early stages of conidiophores of Brazil nut *Aspergillus;* ×500.

PLATE XI

FIG. 33.—Microtome section of normal nut kernel showing tissues named in order, beginning at top: endosperm, epidermis, cortex, procambium, and medulla; ×500.

FIG. 34.—Microtome section of Brazil nut affected by *Pellioniella macrospora* n. sp., showing dense mycelial growth in endosperm region; ×1000.

FIG. 35.—Microtome section of Brazil nut affected by *P. macrospora* n. sp., showing relation of fungus to host tissues; ×500.

FIG. 36.—Germinating conidia of *Actinomyces brasiliensis* n. sp.; ×1000.

FIG. 37.—Hyphae and conidia, *Aciinomyces brasiliensis* n. sp.; ×1000.

FIG. 38.—Vegetative cells of Brazil nut bacillus; ×1000.

FIG. 39.—Colony of Brazil nut bacillus growing near glass in cornmeal agar plate; ×50.

FIG. 40.—Deep colony of Brazil nut bacillus n. sp.; ×50.

FIG. 41.—Surface colony of Brazil nut bacillus n. sp.; ×50.

PLATE XII

FIGS. 42–49, and 51 are of a *Fusarium* which causes dry rot of Brazil nuts.

FIG. 42.—Conidial variation and anastomosis; ×500.

FIG. 43.—Typical hyphae showing difference in size and branching habit; ×500.

FIG. 44.—Hyphae bearing single conidiophores and conidia anastomosed to hyphal cells by germ tube; ×500.

FIG. 45.—Terminal chlamydospores; ×500.

FIG. 46.—Germinating conidia in hanging drop culture; ×500.

FIG. 47.—Anastomosing conidia and hyphae from culture plates; ×500.

FIG. 48.—Anastomosis of germinating conidia in hanging drop; ×500.

FIG. 49.—Conidia and conidiophores of bitter rot fungus, taken from pustules on diseased kernel; ×500.

FIG. 50.—*Phomopsis* conidia, two having germ tubes attached; ×500.

FIG. 51.—Hyphae of *Fusarium* from washed agar plates; ×500.